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Physiological Research on Adventitious Shoot Development in Aspen Roots

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RESEARCH SUMMARY

Adventitious shoots (suckers) from the roots of aspen (*Populus tremuloides* Michx.) originate from newly initiated meristems, preexisting primordia, or suppressed buds. Development of suckers on aspen roots is suppressed by auxin transported from aerial parts. Interference with the auxin supply by cutting, girdling, burning, or herbicide treatments decreases the auxin concentration in the roots enabling suckers to be initiated, or if their growth was suppressed by auxin, to continue to grow. Suckers are probably initiated by cytokinins, which are synthesized in root tips. High cytokinin-auxin ratios favor shoot initiation; low ratios inhibit it. Gibberellins are important in shoot elongation. Any interference with their biosynthesis or action could affect sucker growth. Development of suckers is suppressed on roots of dormant aspen by high levels of dormancy-causing inhibitors such as abscisic acid. Carbohydrate reserves in the parent root supply the energy necessary for bud initiation and shoot outgrowth. An elongating sucker remains totally dependent upon root reserves until it emerges at the soil surface and can carry on photosynthesis.

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INTRODUCTION

Aspen (*Populus tremuloides* Michx.) occurs in clones of a few to thousands of genetically identical individuals. Clonal growth results because aspen has the ability to regenerate vegetatively by adventitious shoots, often called suckers, that arise on its long ropelike lateral roots. Because of short-lived seed and demanding seedbed requirements, aspen seedling establishment is not common. Aspen has been able to remain a widespread and abundant species because of its root suckering ability.

There is probably no other plant species in which single genotypes so completely dominate large areas of land. Although most aspen clones are less than a hectare in size, clones over 10 hectares are not uncommon in some areas. Kemperman and Barnes (1976) delineated a clone in southern Utah that was 43 hectares in size and contained about 47,000 ramets. In areas where the climate enables seedlings to become established, such as the Lake States, clones tend to be smaller. Clone size is probably highly correlated with clone age. However, no method exists by which the age of individual clones can be determined. Possibly some large western clones may have become established during Pliocene or Miocene times, enlarging themselves asexually through many generations of ramets.

Reviewed here are the results of my research on the physiological mechanisms involved in the development of adventitious shoots in aspen roots. Included are the results of some studies that had not been previously published. The data are on file at the Forestry Sciences Laboratory in Logan, Utah.

ORIGIN OF SUCKERS

In many woody plants that have the ability to regenerate by root suckers, the suckers originate from

preexisting suppressed buds that arise during normal development of primary tissue systems (Schier and Campbell 1976). The buds are embedded in the periderm and their vascular traces extend to the primary xylem of the stele. Unlike most other species, root suckers in aspen do not originate at a specific time in the ontogeny of a root. Instead, they develop from meristems that appear to be initiated near the cork cambium at various times during secondary growth (Brown 1935; Sandberg 1951; Schier 1973b). These meristems may develop into buds and then elongate into shoots, but frequently growth is arrested at the primordial stage or after the formation of a bud. Development may not be continuous because the physiological requirements for the initiation of an apical meristem are different from that for its growth and extension (Butenko 1964; Schier 1973a).

Thousands of suppressed shoot primordia can be found on the roots of most aspen clones. They occur as small mounds protruding from the cork cambium. The primordia can be seen without magnification by peeling off the cork (Schier 1973b). Primordia occur in various stages of ontogeny: from those that are essentially small masses of meristematic cells with no tissue differentiation, to those in which procambium and protoxylem elements are differentiated. The length of time an adventitious meristem can remain in the primordial stage is unknown.

Although suckers do arise from suppressed buds, buds are not as important a source of suckers as are newly initiated meristems or preexisting primordia (Sandberg 1951; Schier 1973b). Sandberg (1951) observed that suppressed buds on roots often remained inhibited, while numerous newly initiated meristems on the same root developed into suckers. Suckers that originated from suppressed buds elongated much less vigorously than those that were recently initiated.

INTERCLONAL AND INTRACLONAL VARIATION

In the relative capacity of aspen clones to produce suckers, large clonal differences have been found when suckers are propagated from root cuttings under controlled environmental conditions (table 1) (Schier 1974). The magnitude of the differences among clones varies with the date of collection because the seasonal trend in sucker production shows considerable clonal variation. The number of suckers produced by a clone is probably determined by the balance of food reserves and hormonal growth regulators in the roots. Genotype probably has a large influence on suckering capacity, but nongenetic factors such as clone history, stem age, and environmental variables could also be major contributors. Some clones, in spite of a high suckering capacity, produce few viable suckers because their excised roots are highly susceptible to decay (table 2). The season when roots are collected has a significant effect on the incidence of decay during sucker propagation.

Within an aspen clone exists considerable variation in suckering capacity among lateral roots (table 3) (Schier 1978a). Differences among lateral roots are probably caused by differences in the physiological condition of the roots (such as, water content, hormone levels and ratios, concentration of nutrients), which in turn is caused by microclimate variability and root position in the clonal root system. Temperature, an important microclimate element, varies with soil depth and exposure to radiation. Physiological condition as controlled by root position depends upon proximity and attachment to trees of various ages and vigor. This position will determine the quantity of photosynthates and auxins and other growth regulators translocated to a particular root.

There is no evidence of a gradient in suckering capacity in a segmented root; that is, cuttings from a root do not show a significant change in suckering capacity with increasing distance from the stem (Schier 1978a). This indicates that distance from the parent tree or root age are not factors regulating suckering within lateral roots.

Table 1.—Mean suckering ability of root cuttings (10 × 1 to 2 cm) from 20 Utah aspen clones collected on 3 different dates after 6 weeks in moist vermiculite (greenhouse temperatures 15-25° C)

| Clone | Suckers (>5 mm) per segment | | | | Height of tallest sucker (mm) | | | |
|-------|-----------------------------|------|------|---------|-------------------------------|------|------|---------|
| | 5/76 | 7/75 | 9/74 | Average | 5/76 | 7/75 | 9/74 | Average |
| 1 | 10.5 | 6.0 | 9.2 | 8.57 | 29 | 26 | 31 | 28.7 |
| 2 | 4.9 | 2.7 | 6.0 | 4.53 | 39 | 33 | 34 | 35.3 |
| 3 | 5.9 | 6.1 | 4.5 | 5.50 | 37 | 30 | 33 | 33.3 |
| 4 | 2.4 | 5.5 | 5.0 | 4.30 | 46 | 41 | 34 | 40.3 |
| 5 | 4.4 | 10.7 | 3.4 | 6.17 | 35 | 30 | 24 | 29.7 |
| 6 | 7.3 | 8.7 | 7.3 | 7.77 | 47 | 42 | 44 | 44.3 |
| 7 | 6.9 | 7.5 | 7.1 | 7.17 | 38 | 32 | 44 | 38.0 |
| 8 | 14.0 | 21.4 | 13.3 | 16.23 | 28 | 26 | 32 | 28.7 |
| 9 | 6.7 | 12.7 | 21.4 | 13.60 | 36 | 31 | 37 | 34.7 |
| 10 | 4.5 | 3.6 | 16.5 | 8.20 | 32 | 30 | 35 | 32.3 |
| 11 | 5.9 | 3.4 | 5.0 | 4.77 | 29 | 21 | 30 | 26.7 |
| 12 | 8.4 | 8.2 | 9.3 | 8.63 | 39 | 27 | 42 | 36.0 |
| 13 | 2.2 | 2.1 | 4.1 | 2.80 | 37 | 22 | 30 | 29.7 |
| 14 | 6.5 | 15.3 | 9.5 | 10.43 | 46 | 27 | 44 | 39.0 |
| 15 | 5.0 | 5.6 | 3.9 | 4.83 | 35 | 36 | 49 | 40.0 |
| 16 | 11.1 | 15.4 | 21.2 | 15.90 | 47 | 30 | 36 | 37.7 |
| 17 | 7.9 | 8.3 | 14.6 | 10.27 | 38 | 23 | 32 | 31.0 |
| 18 | 6.2 | 9.8 | 4.6 | 6.87 | 28 | 30 | 44 | 34.0 |
| 19 | 6.0 | 6.0 | 6.5 | 6.17 | 36 | 29 | 35 | 33.3 |
| 20 | 6.1 | 3.1 | 4.6 | 4.60 | 32 | 30 | 35 | 32.3 |
| Mean | 6.64 | 8.1 | 8.85 | 7.87 | 36.7 | 29.8 | 36.2 | 34.2 |

Table 2.—Percentage of aspen root segments (10 × 1 to 2 cm) showing signs of decay after 6-weeks in moist vermiculite in a growth chamber (25°/15° C, 12/12 h temperature regimen)

| Collection date | Clone | | | | | | | | | | Mean |
|-----------------|-------|----|----|---|----|---|---|---|----|----|------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| June | 48 | 72 | 55 | 5 | 8 | 0 | 0 | 2 | 5 | 0 | 20 |
| August | 45 | 75 | 35 | 2 | 12 | 0 | 0 | 0 | 12 | 2 | 19 |
| October | 12 | 48 | 20 | 5 | 0 | 0 | 5 | 0 | 0 | 2 | 9 |
| Mean | 35 | 65 | 37 | 4 | 7 | 0 | 2 | 1 | 6 | 2 | 16 |

Table 3.—Mean suckering ability of root segments (10 cm) excised from 27 randomly selected lateral roots (diameter, 1 to 2 cm; length, 51 to 412 cm) from a single aspen clone after 6-weeks in moist vermiculite (greenhouse temperatures, 15-25° C)

| Lateral root | Suckers (>5 mm) per segment | Height of tallest sucker (mm) | Lateral root | Suckers (>5 mm) per segment | Height of tallest sucker (mm) |
|--------------|-----------------------------|-------------------------------|--------------|-----------------------------|-------------------------------|
| 1 | 6.3 | 38 | 15 | 6.1 | 42 |
| 2 | 3.7 | 48 | 16 | 4.8 | 37 |
| 3 | 11.6 | 51 | 17 | 8.3 | 42 |
| 4 | 8.6 | 41 | 18 | 11.6 | 51 |
| 5 | 7.8 | 45 | 19 | .8 | 17 |
| 6 | 4.0 | 35 | 20 | 9.6 | 45 |
| 7 | 11.2 | 41 | 21 | .6 | 12 |
| 8 | 13.0 | 42 | 22 | 7.8 | 38 |
| 9 | 10.2 | 30 | 23 | 10.7 | 51 |
| 10 | 17.2 | 40 | 24 | 3.4 | 48 |
| 11 | 15.9 | 49 | 25 | 12.3 | 35 |
| 12 | 4.9 | 42 | 26 | 6.8 | 49 |
| 13 | 5.2 | 43 | 27 | 22.7 | 44 |
| 14 | 4.1 | 37 | | | |
| | | | Mean | 8.56 | 42.0 |

ROLE OF GROWTH REGULATORS

Adventitious shoot development on aspen roots is stimulated by cutting stems. We are dealing with a form of apical dominance, a term traditionally used to describe the control exerted by the shoot apex over lateral buds. Substantial evidence shows that sucker development is suppressed by auxin transported from aerial parts (Farmer 1962; Eliasson 1971a, 1971b, 1972; Schier 1973d, 1975). Less is known about the factors that induce shoot initiation, although research in other plants and the culture of plantlets in aspen callus indicate that cytokinins synthesized in roots are likely to be involved (Peterson 1975; Winton 1968; Wolter 1968). High cytokinin-auxin ratios probably favor shoot initiation and low ratios inhibit it. Other hormones studied because of their possible role in sucker development are abscisic acid, ethylene, and the gibberellins.

Auxins

I used three approaches to determine if root suckering is inhibited by auxin:

1. Determine the effect of indole-3-acetic acid (IAA), a naturally occurring auxin, on suckering from root cuttings.
2. Determine if there is a relationship between sucker production and levels of endogenous auxin.
3. Determine if suckering can be stimulated by treating roots with an antiauxin.

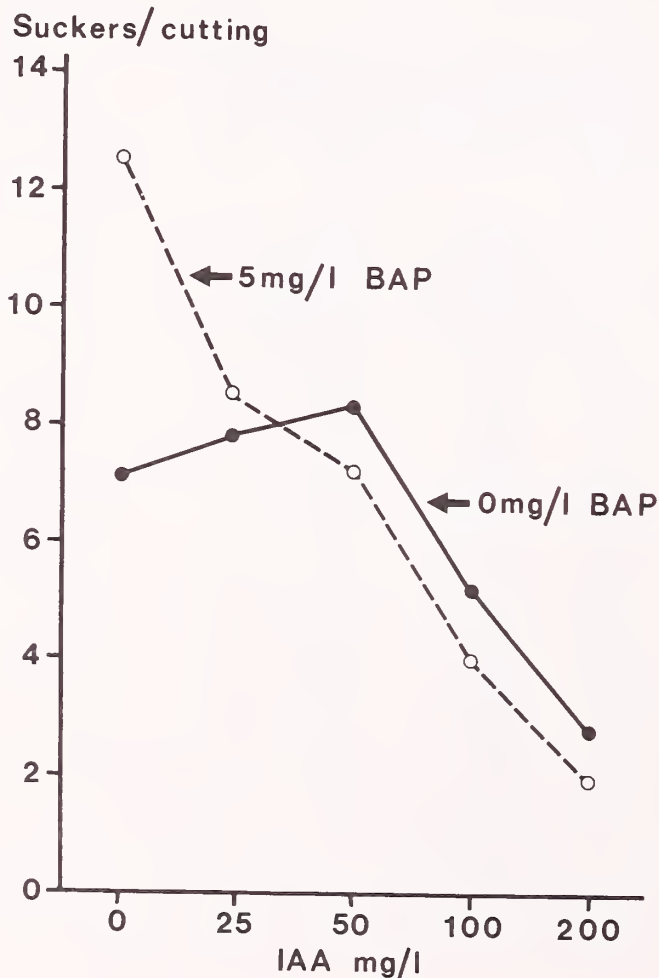


Figure 1.—Effect of five concentrations of IAA with and without 5 mg/l BAP on suckering from aspen root cuttings.

The inhibitory effect of exogenous auxin on sucker production was shown by treating root segments with a range of concentrations of IAA with and without 5 mg/l of the cytokinin 6-benzylamino-purine (BAP) (fig. 1).¹ By itself, IAA became inhibitory at concentrations over 50 mg/l. BAP alone increased sucker production over that of the controls by 78 percent. Its promotive effects were entirely eliminated by adding as little as 25 mg/l IAA. At high IAA concentrations, BAP appeared to interact synergistically with IAA, increasing its inhibitory action, but there was no significant difference between the paired treatments.

The inhibitory action of auxin on sucker development was also tested by treating root segments with a single concentration of IAA (100 mg/l) in combination with varying concentrations of BAP (fig. 2). BAP alone stimulated sucker production showing its greatest promotion at 10 mg/l. Addition of IAA reduced sucker production below that of the controls and eliminated any promotive effects of BAP.

Eliasson (1971b), studying suckering in *Populus tremula*, found that removal of growing shoot parts from young plants reduced the concentration of auxin in the roots. He also found that auxin levels in root cuttings decreased during the first 24 hours after excision due to its rapid destruction, and was low during the period of sucker development (Eliasson 1971a). Transport of auxin to roots must be continuous if inhibitory levels of auxin are to be maintained. However, even after much auxin has been inactivated, there probably is still enough remaining in root cuttings to affect sucker production (Schier 1973d).

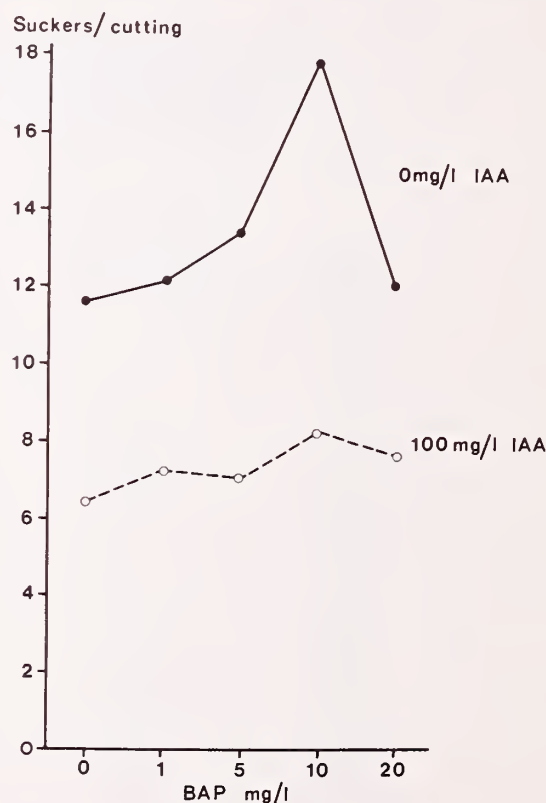


Figure 2.—Effect of five concentrations of BAP with and without 100 mg/l IAA on suckering from aspen root cuttings.

¹The standard procedure for testing the effects of growth regulators on sucker production was to soak root cuttings (10 cm in length, 1 to 2 cm in diameter) for 24 h in aqueous solutions of the chemicals and then to plant them horizontally in moist vermiculite. After 6 weeks, the number of shoots over 5 mm in height were determined.

This was indicated in a study of the relationship, between the seasonal variation in sucker production from root cuttings of three aspen clones and concentrations of auxin in the roots when collected (fig. 3). A decrease in sucker numbers was correlated with an increase in auxin concentration (fig. 4). A regression developed by combining the data of the three clones was significant. After the variance due to clones was accounted for, 72 percent of the variation in sucker numbers was associated with auxin content. The fewest suckers were produced in June during the flush in shoot growth when the greatest quantity of auxin was found in the roots.

Thus it appears that there is enough auxin in root cuttings to affect initiation and early development of suckers. Auxin inactivation probably causes a change from a high level of inhibition in the intact plant to a low level in root segments.

The possibility exists that auxin may not be destroyed, but converted to indoleacetyl aspartic acid (personal communication, Kenneth V. Thimann, University of California, Santa Cruz). Later, under modified circumstances, this peptide may be slowly hydrolyzed to provide a source of auxin in root segments.

Even though a good correlation existed between seasonal variation in sucker production and concentration of endogenous auxin, a causal relationship was not shown. Therefore, the hypothesis that auxin in excised roots at the time of collection inhibits subsequent sucker development, was tested by treating root segments with an apparent antiauxin, α -(*p*-chlorophenoxy)isobutyric acid (CPIBA). This was an attempt to reduce auxin inhibition and thereby increase sucker production (Schier 1975). Cuttings were collected in the spring when endogenous auxin levels were highest. The antiauxin significantly increased sucker production (fig. 5). Number of suckers on cuttings treated with 25 mg/l CPIBA was twice the number produced by controls. Thus, CPIBA apparently had relieved auxin inhibition.

Additional evidence that auxin in excised roots affects suckering was indicated by the polarity shown in sucker production in root sections (Schier 1978a). A gradient was found in the number of suckers arising along uncut 50 cm root sections decreasing from the proximal to the distal ends (fig. 6). Polarity in roots is usually attributed to the movement of endogenous auxin to the distal ends (Robinson and Schwabe 1977). When the root sections were cut into 10 cm segments, a polar gradient in sucker numbers failed to develop along the section because auxin could not be redistributed. However, there was a gradient in each individual 10 cm segment: 64 percent of the suckers arising on the proximal halves and only 36 percent on the distal halves.

Once suckers develop on roots, transport of auxin from elongating shoots may increase auxin levels in the parent roots and inhibit further sucker initiation (Schier 1972).

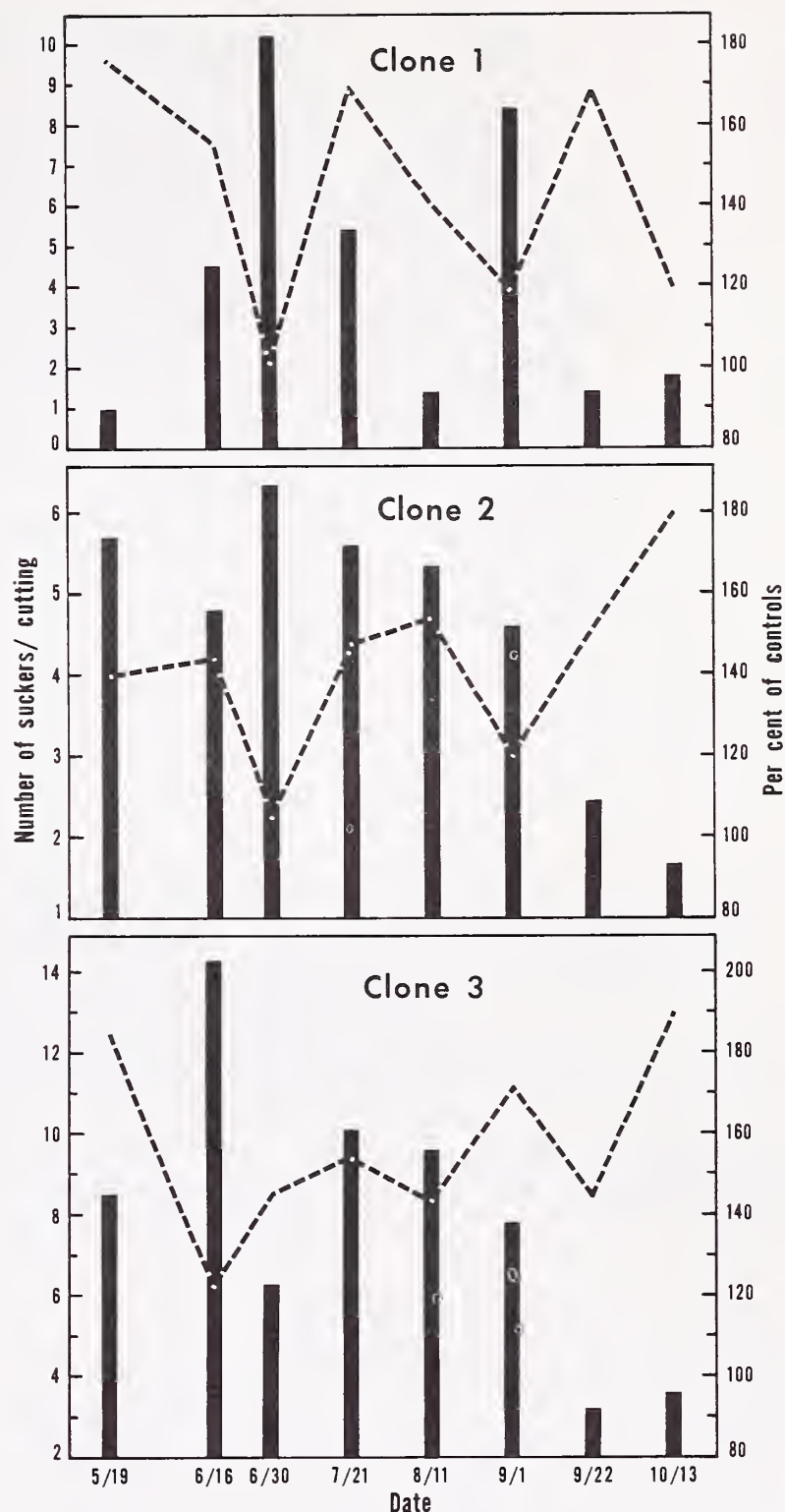


Figure 3.—Seasonal variation in auxin content of roots at time of collection (solid vertical bars) and average number of suckers subsequently produced from root cuttings (dashed horizontal lines) for three aspen clones. Auxin-like activity was measured by the average elongation in percentage of controls of *Avena* internode sections, which were incubated with the IAA zone of chromatograms (R_f 0.2-0.3) of the acid ether fraction of methanol extract from root bark.

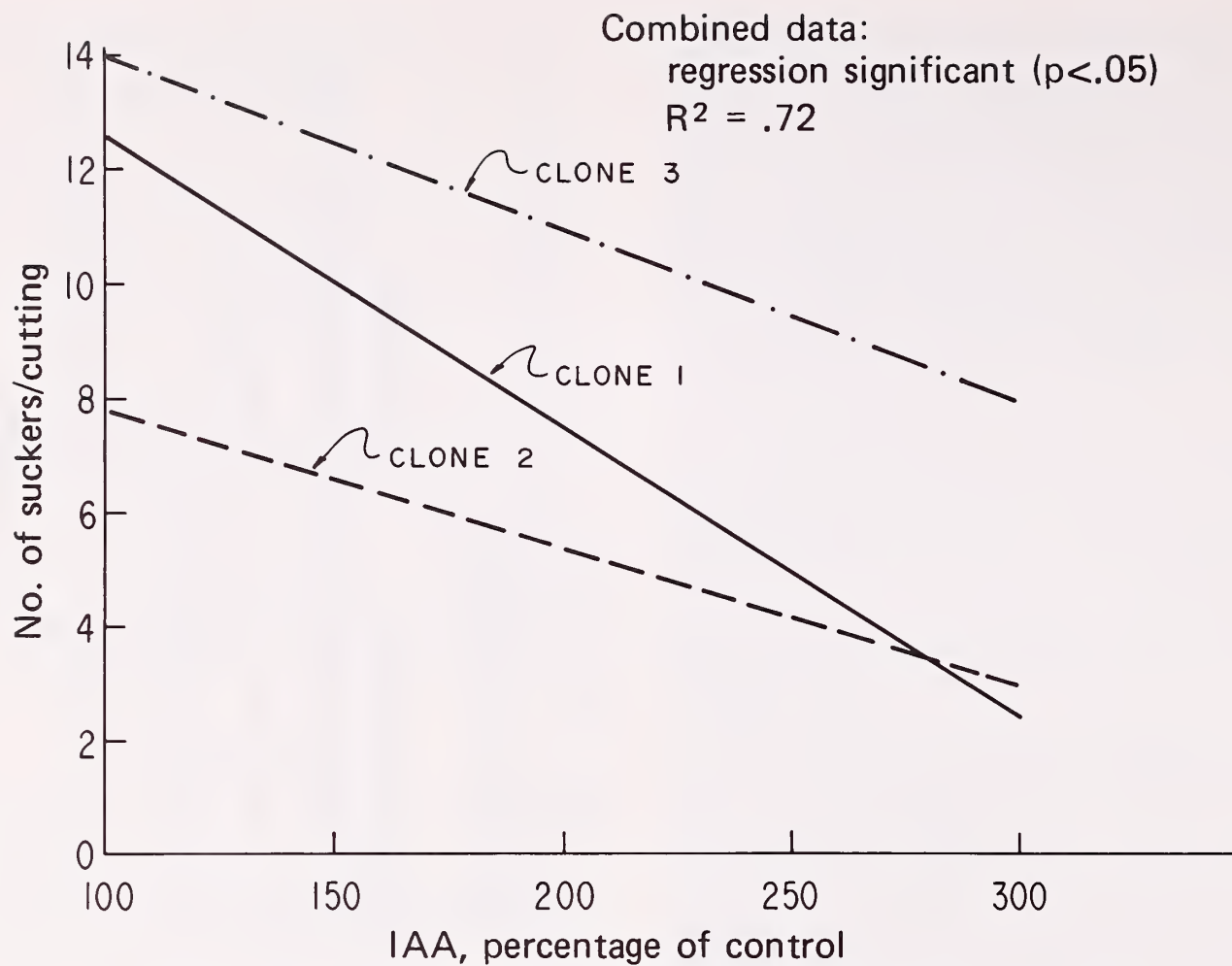


Figure 4.—Relationship between the concentration of auxin in root bark of three aspen clones when collected and number of suckers subsequently produced by root cuttings.

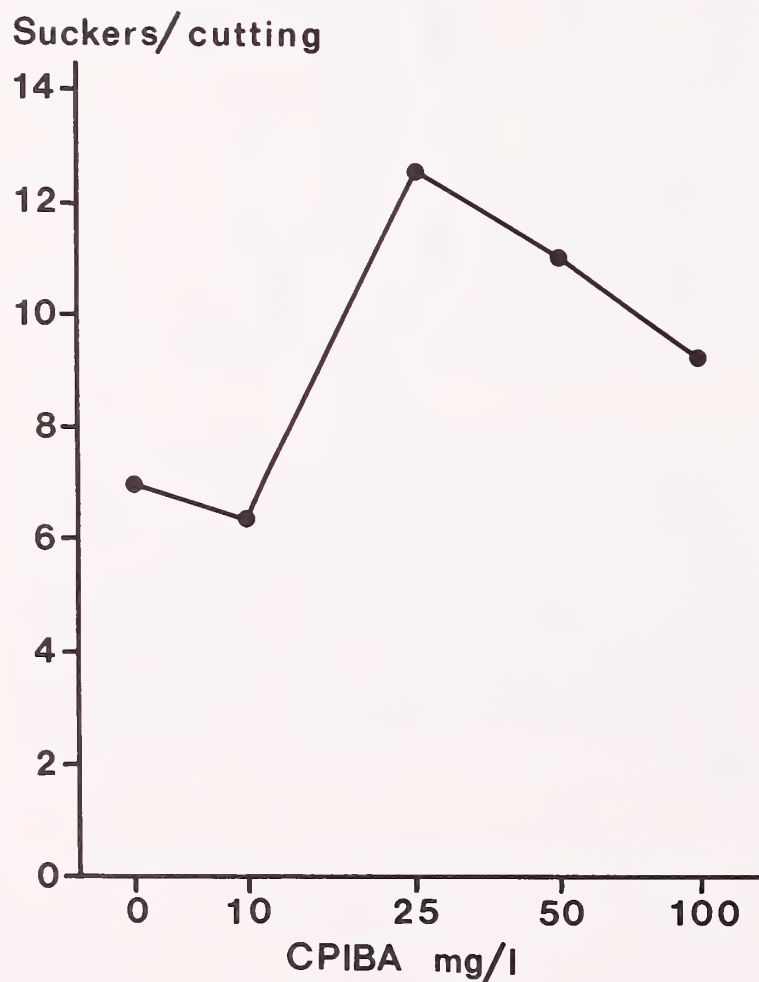


Figure 5.—Effects of the antiauxin, α -(*p*-chlorophenoxy)isobutyric acid (CPIBA), on sucker production by aspen root cuttings.

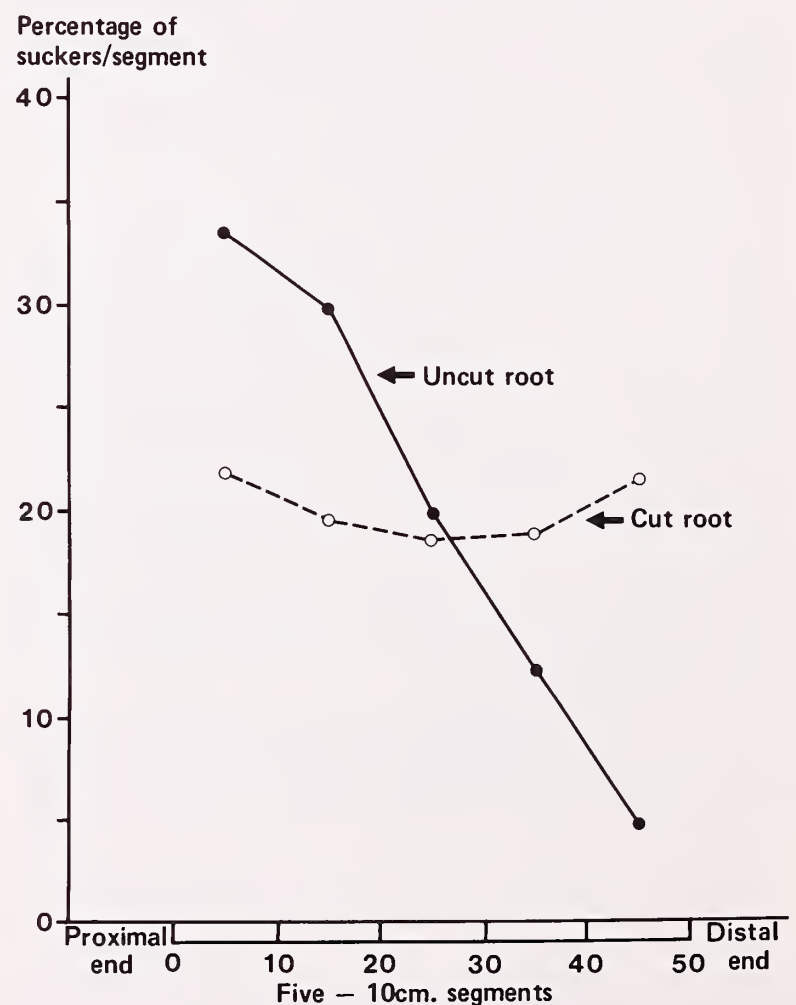


Figure 6.—Distribution of suckers along cut and uncut 50-cm sections of aspen roots.

This is supported by experiments in which continuous shoot removal increased sucker production from root cuttings.

The relatively large number of suckers that appear to arise regularly in many undisturbed aspen clones indicates that apical dominance is not absolute (Schier 1975; Schier and Smith 1979). This is not surprising when one considers the distance IAA, a relatively unstable compound, must be transported from its source in developing buds and young leaves to the roots where it brings about its morphogenetic effects. Apical dominance weakens as auxin goes down the stem due to immobilization, destruction, and age effects (Thimann 1977).

During normal seasonal tree growth, there are periods when apical dominance is weak enough for suckering to occur. For example, in spring before bud burst and translocation of auxin to the roots, temperatures are often high enough for sucker initiation and growth (Schier 1978b). Sucker formation is inhibited after the leaf buds have opened and apical dominance is reasserted.

Cytokinins

Considerable evidence in the literature shows that cytokinins have an important role in shoot initiation in roots (Peterson 1975) and in the release of buds from apical dominance (Thimann 1977). Several cytokinin-like compounds have been isolated from a poplar hybrid (Hewett and Wareing 1973). The promotive effects of BAP on sucker production by root cuttings are shown in figures 1 and 2. However, in numerous experiments in which the effects of natural and synthetic cytokinins were tested, I failed to get a response to cytokinin treatments. The reason might be that endogenous cytokinins in the roots were at optimum levels for sucker formation at the time of treatment. One of my early experiments supports this conclusion (Schier 1970). I treated root cuttings with kinetin, dissolving it in 0.05 N HCl (pH 1.2). This acidic solvent expectedly was highly inhibitory, but it did lead to some interesting results (fig. 7). Relative to the distilled water control, the solvent alone reduced sucker production by 84 percent. However, this inhibition was overcome by the addition of kinetin. A logical interpretation of these results is that the solvent caused a breakdown of endogenous cytokinins essential for sucker development, and the need for a cytokinin was replaced by kinetin.

I also haven't had much success in stimulating adventitious shoot development in intact and decapitated hydroponically grown aspen by treatment with cytokinins (Schier 1977). This was due to the toxicity of relatively low concentrations. Nutrient solutions containing 1.6 mg/l 6-benzylamino-purine (BAP) caused death of half of the plants and leaf-yellowing and -browning in others. All plants growing in nutrient solutions containing 6.4 mg/l BAP were dead within 7 days of adding the cytokinin to the solution. Severe drought symptoms and mortality were caused by root-dieback. At lower concentrations of BAP, drought symptoms were not observed, but water absorption by the roots was reduced as indicated by a decrease in transpiration. Collins and Kerrigan (1974) reported that cytokinins reduce the water permeability

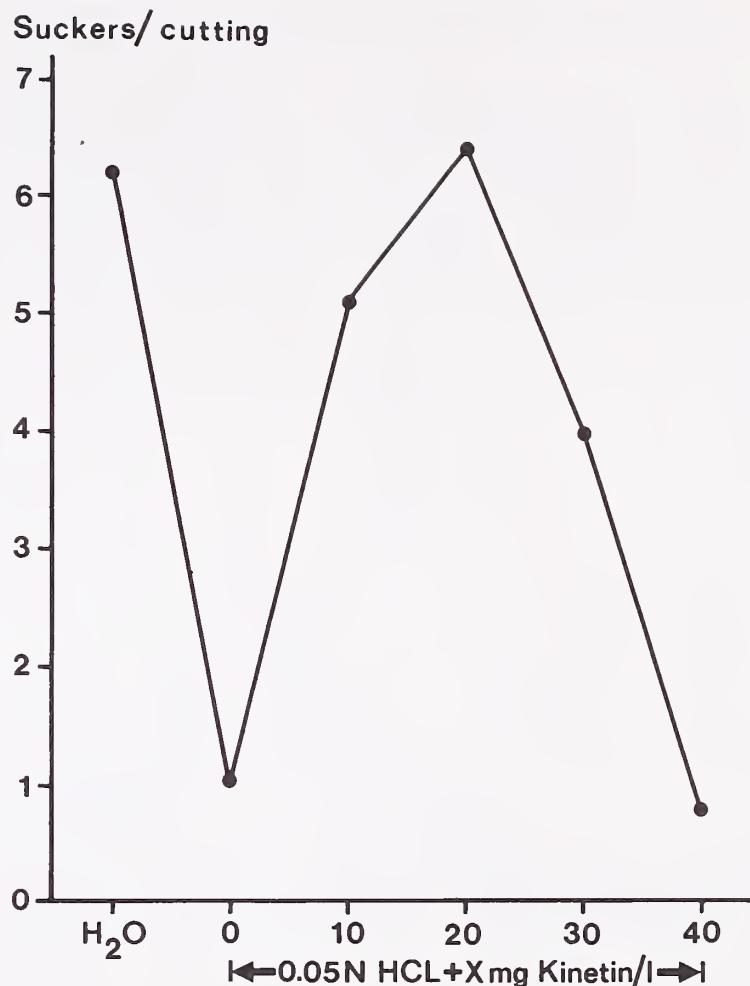


Figure 7.—Effects of kinetin on the inhibiting effect of HCl on aspen root suckering.

of plant roots. Root buds were found in only a few plants treated with 0.4 mg/l BAP. These were confined to parts of roots near the base of the stem.

Gibberellins

Schier and others (1974) found at least three gibberellin-like compounds in the methanolic extract of aspen roots. The main peak of biological activity co-chromatographed with gibberellic acid (GA₃) on silica gel partition columns (fig. 8). A mass peak with the same retention time as GA₃ was present on two gas-liquid chromatography columns. Concentration of the gibberellins in roots of mature trees varied with the season of the year. Levels remained high after leaf fall at which time auxins could no longer be detected.

The effect of GA₃ on suckering from excised roots depends on the stage of development of the adventitious shoots when the roots are treated (Schier 1973a). If the shoots arise primarily from newly initiated meristems (clone 8, fig. 9), GA₃ reduces sucker production because it inhibits initiation and division of the first shoot primordium cells (Heide 1969; Murashige 1964). As a primordium increases in cell number, GA₃ shows progressively less inhibition of bud formation. At some stage in bud development, GA₃ will stimulate emergence of established shoot primordia. Therefore, GA₃ treatment will promote sucker production from root segments having large well-developed primordia (clone 13, fig. 9).

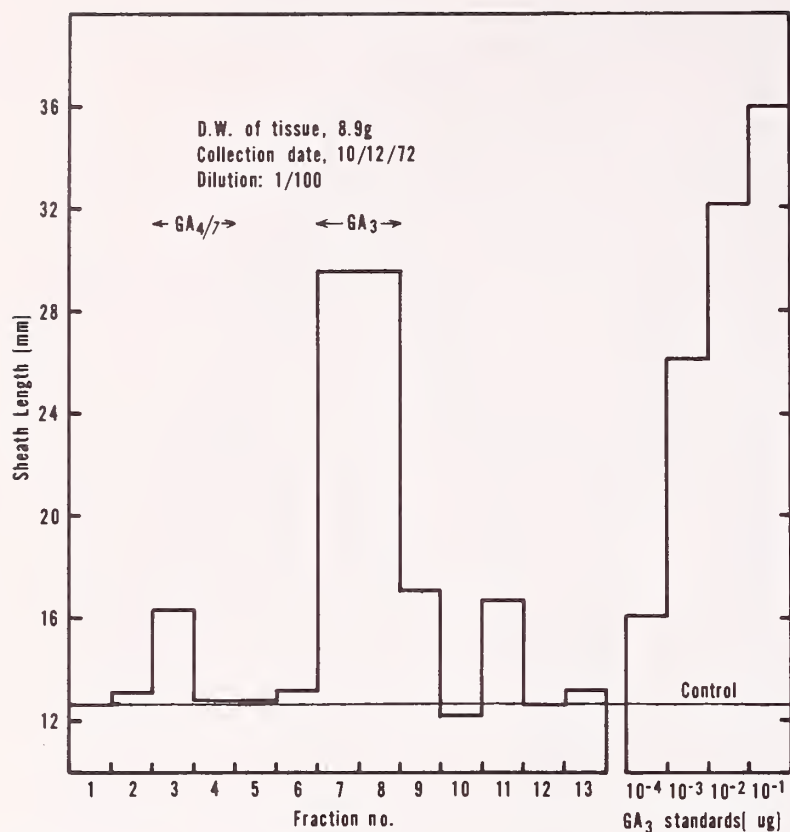


Figure 8.—Dwarf rice (cv. Tan-ginbozu) microdrop assay of a silica gel partition column chromatogram of a purified methanol extract of aspen roots.

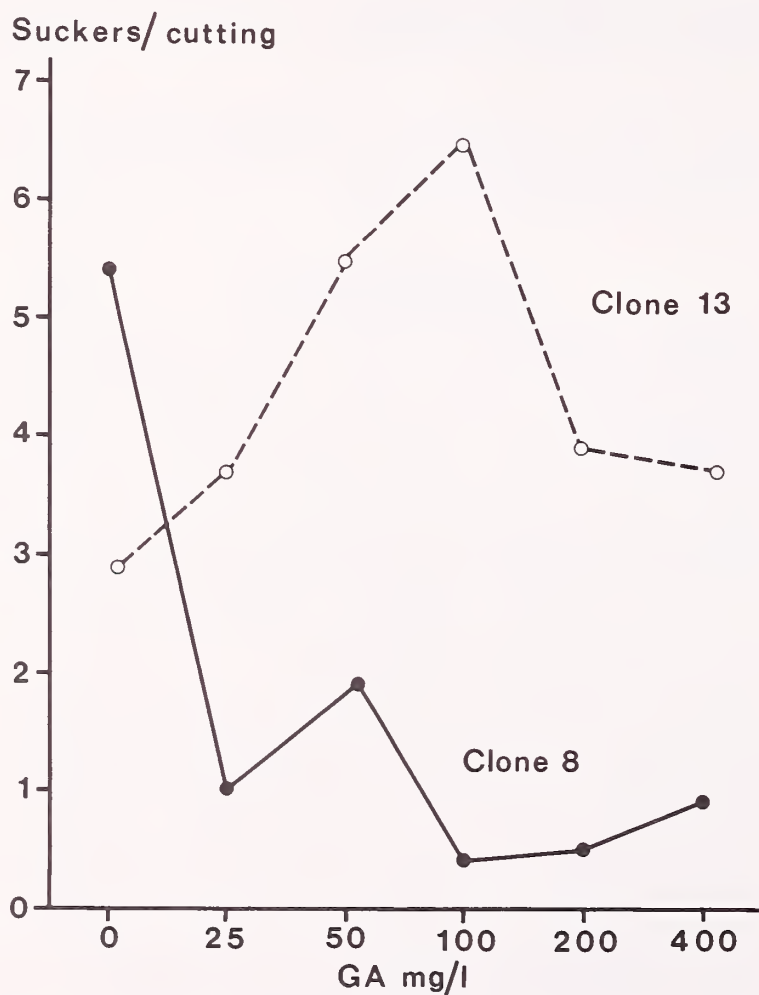


Figure 9.—Effects of gibberellic acid (GA₃) on sucker production by aspen root cuttings with (clone 13) and without (clone 8) well-developed preexisting shoot primordia.

The role of endogenous gibberellin in sucker development was studied by treating roots having numerous preexisting shoot primordia with A-rest (a substituted pyrimidine, formerly EL-531), the trade name for a growth regulator manufactured by Elanco Products Co. known to inhibit the role of gibberellin in internode elongation (Leopold 1971). A-rest inhibited sucker production suggesting that endogenous gibberellins are important in sucker growth (fig. 10).

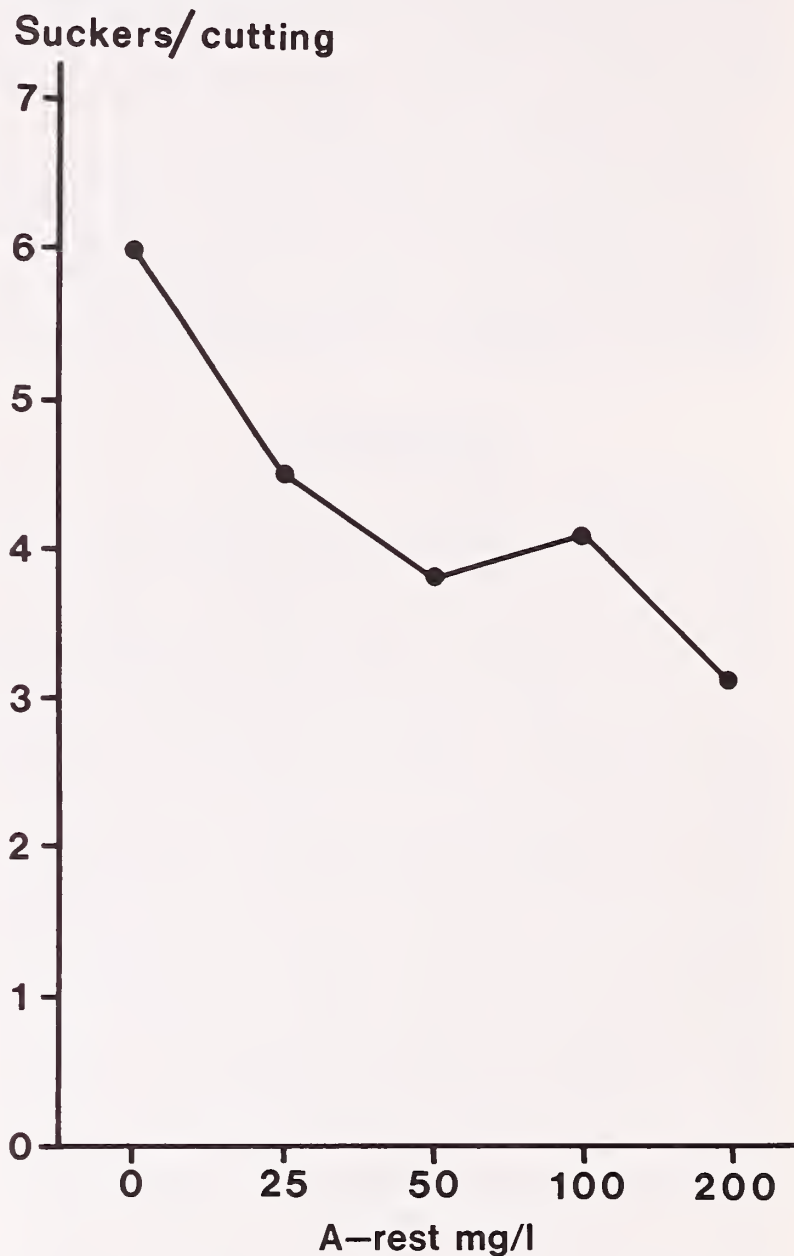


Figure 10.—Effects of A-Rest (formerly EL-531) on sucker production by aspen root cuttings.

Absciscic Acid

Although abscisic acid (ABA) is a well-known growth inhibitor, Heide (1968) found that it promoted bud initiation in detached leaves of Begonia. Therefore, I was interested in the effect ABA would have on sucker development (Schier 1973c). If ABA was found to be inhibitory, it would be interesting to test the ability of GA₃ to reverse ABA inhibition. Root cuttings were treated with ABA at the start of the experiment. At 26 days the cuttings were lifted and half treated with 100 mg/l GA₃, half with distilled water. Then they were replanted. All cuttings were examined at the end of 40 days.

ABA inhibited sucker production (fig. 11). Following ABA, treatment with GA₃ resulted in partial reversal of the ABA inhibition. A growth retardation of about 30 percent remained that could not be reversed by application of GA₃. Since GA₃ is known to be inhibitory during the early stages of sucker development (Schier 1973a), the 30 percent retardation may represent the relative number of primordia that ABA prevented from developing to a stage at which they could be promoted rather than inhibited by GA₃.

ABA may have a role in inhibiting sucker growth in dormant aspen. When young aspen were decapitated after going dormant in late summer, buds formed on the roots but they did not elongate until the following spring (Schier 1978b). Regulation of dormancy generally seems to be controlled by a balance between endogenous inhibitors, such as ABA, and growth promoting substances, especially gibberellins. Dormancy is broken by low winter temperatures, which lowers the inhibitor: growth-promoter ratio.

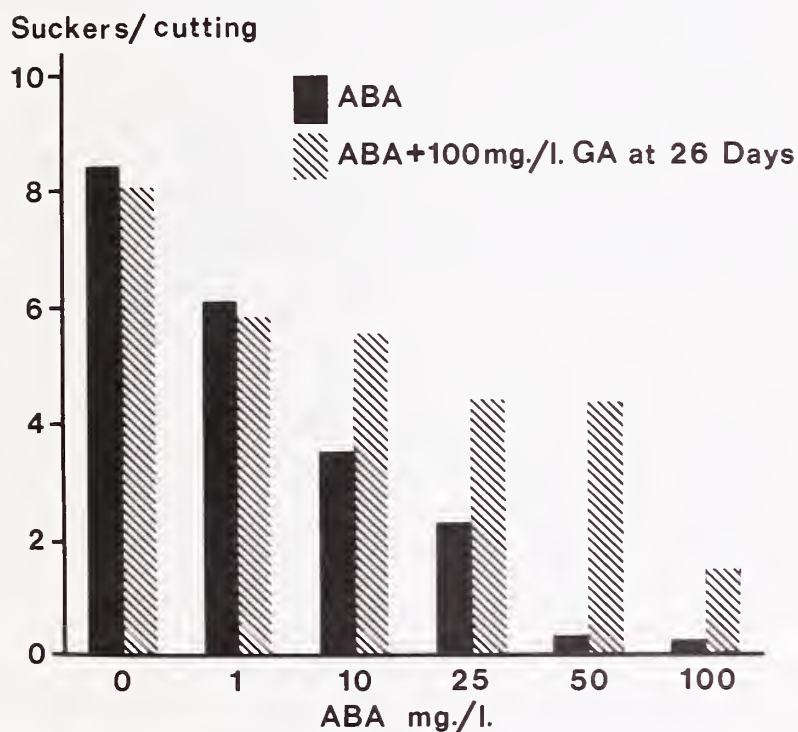


Figure 11.—Effects of *cis-trans* ABA and GA₃ on sucker production by aspen root cuttings. Cuttings were treated with six concentrations of ABA and then planted. After 26 days, the cuttings were lifted, treated with 100 mg/l GA₃, and replanted. Fourteen days later sucker counts were made on all cuttings.

Ethylene

Ethylene affects a wide range of developmental processes in plants, including abscission, apical dominance, adventitious bud development, rooting, and senescence. Auxin-induced ethylene production could account for the inhibitory action of applied auxin (Burg 1973; Abeles 1973). Therefore, I thought it worthwhile to test the effect of this hormone on sucker development.

Ethephon [(2-chlorethyl) phosphonic acid] is an effective ethylene generator and was a convenient agent for bringing about ethylene responses in aspen roots. The effect of ethephon on adventitious shoot development was similar on cuttings collected in June and August from the same clone (fig. 12) (Schier and Campbell 1978). At 100 mg/l, ethephon significantly increased shoot production relative to controls. Further increases in concentration resulted in a decrease in sucker production.

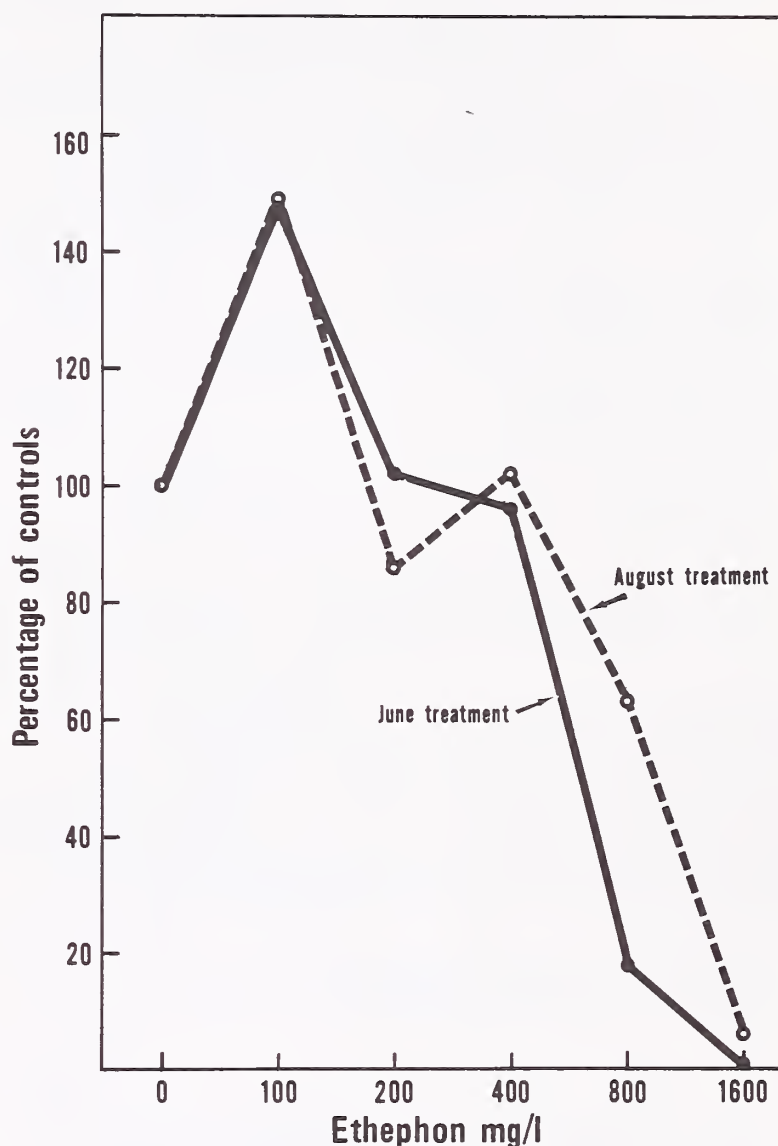


Figure 12.—Effect of ethephon (Amchem's Ethrel formulation 68-240) on sucker production by aspen root cuttings collected in June and August 1974. The mean number of suckers in each treatment is expressed as the percentage of suckers on control cuttings. Mean number of suckers per cutting in controls: June, 8.48 and August, 5.08.

Suppression of suckering by high concentrations of ethephon may have resulted because the ethylene generated is an inhibitor of cell division. However, the gas also has the ability to reduce inhibition caused by endogenous auxin. Thus, there was a potential for growth promotion after the ethylene dissipated. Apparently, this is why ethephon at 100 mg/l stimulated shoot production. At the higher concentrations, ethylene probably remained at inhibitory levels during the entire propagation period.

ROLE OF FOOD RESERVES

In addition to growth regulators, the energy supplied by carbohydrates and other reserves is necessary for sucker initiation and growth. Suckers arise from parent roots that are as deep as 30 cm in the soil. Elongating suckers will remain dependent on reserves until they can emerge from the soil, develop leaves, and carry on photosynthesis.

Carbohydrates

In a study of the role of carbohydrate reserves in roots of Alaskan and Utah aspen at various seasons on initial sucker growth, suckers were propagated in darkness in order to simulate light conditions in soil (Schier and Zasada 1973). A seasonal variation in percentage of total nonstructural carbohydrates (TNC) was found in aspen roots (fig. 13). The trend was similar in both areas. Carbohydrate contents of the roots showed a significant relationship to both clone of origin and date of collection.

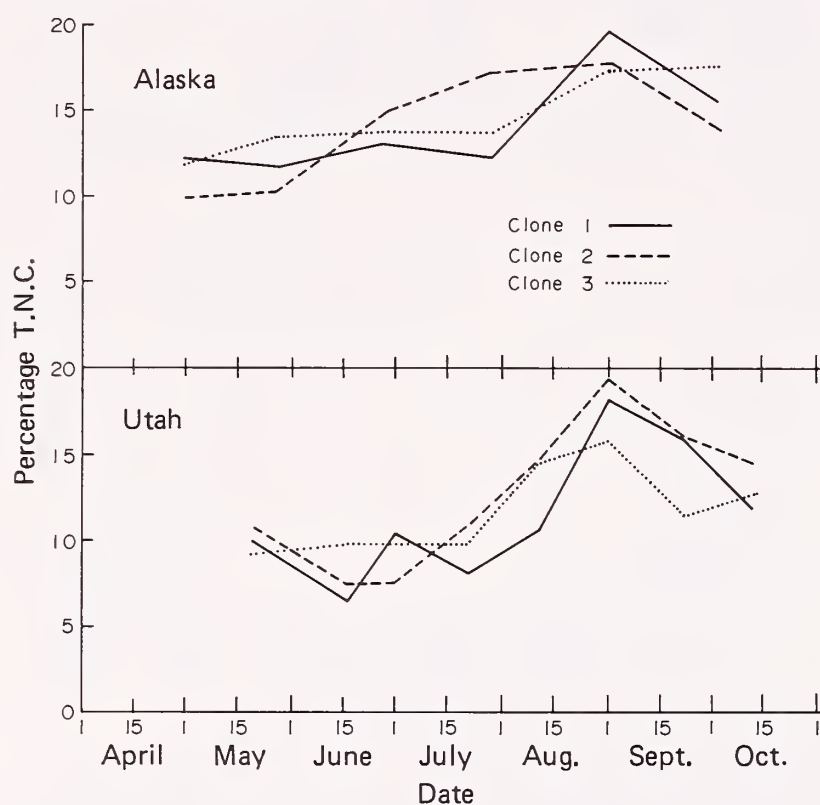


Figure 13.—Seasonal variation in percentage of total nonstructural carbohydrates (T.N.C.) in aspen roots from Utah and Alaska.

For the range in concentration of TNC in root samples, 5 to 20 mg/g dry weight, there was no correlation between sucker numbers and carbohydrate content. Numbers of suckers appear to be a function of hormone levels and ratios. However, in the additive models that were constructed from the data (fig. 14), carbohydrate reserves contributed significantly both statistically and quantitatively to sucker dry weight. As shown in figure 14A, dry weight of all suckers produced on a cutting was positively correlated with both number of suckers per cutting and percentage of total nonstructural carbohydrates. The effect of sucker numbers

indicates that utilization of reserve carbohydrates is dependent upon the number of growing apices or sinks for metabolites. The larger the number of elongating shoots, the greater the capacity of sucker growth to tap the pool of reserve carbohydrates.

Figure 14B indicates that although dry weight of all suckers per cutting increased as the number of suckers became larger, dry weight of each individual sucker decreased. This is probably due to correlative inhibition and competition among suckers for a limited supply of carbohydrates.

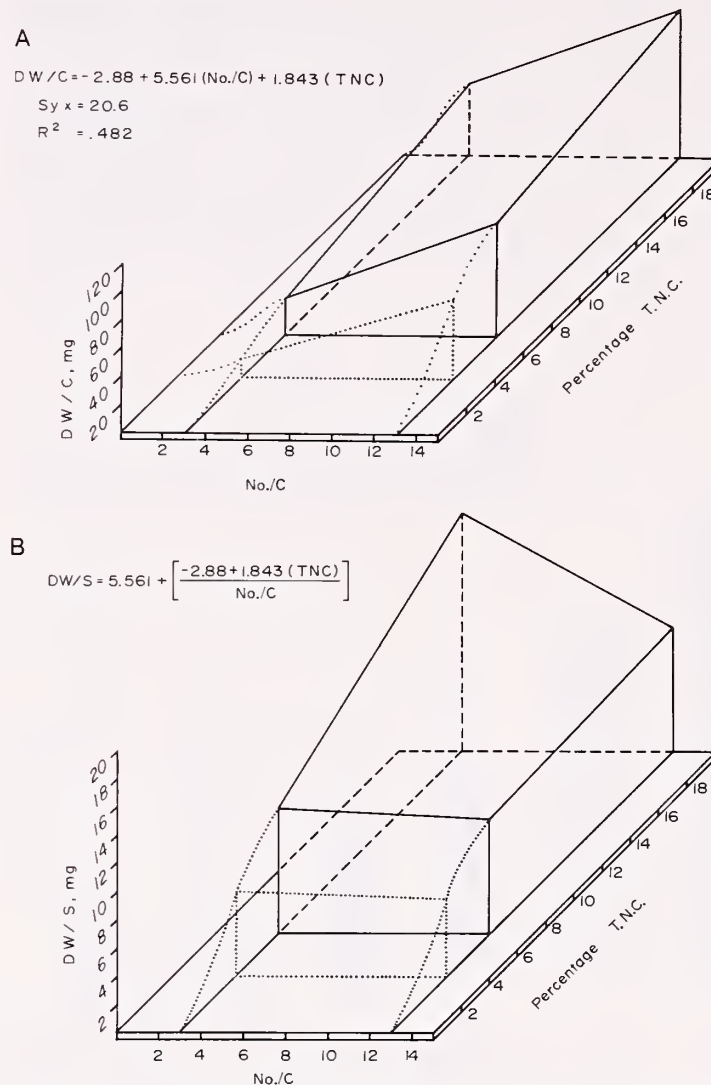


Figure 14.—Additive models in which (A) dry weight of suckers per cutting (DW/C) and (B) dry weight per sucker (DW/S) are a function of number of suckers per cutting (No./C) and percentage of total nonstructural carbohydrates (T.N.C.). The models were constructed using the combined data from each of three Utah aspen clones. Dotted lines are extrapolated.

Nitrogenous Compounds and Lipids

The role of total nitrogen and lipids (saponifiable) in aspen roots on initial development of suckers was investigated in a single clone. Neither numbers nor dry weight of suckers were correlated with the seasonal variation in concentration of these nutritional factors. Concentrations of total nitrogen and lipids were significantly related to date of collection (fig. 15).

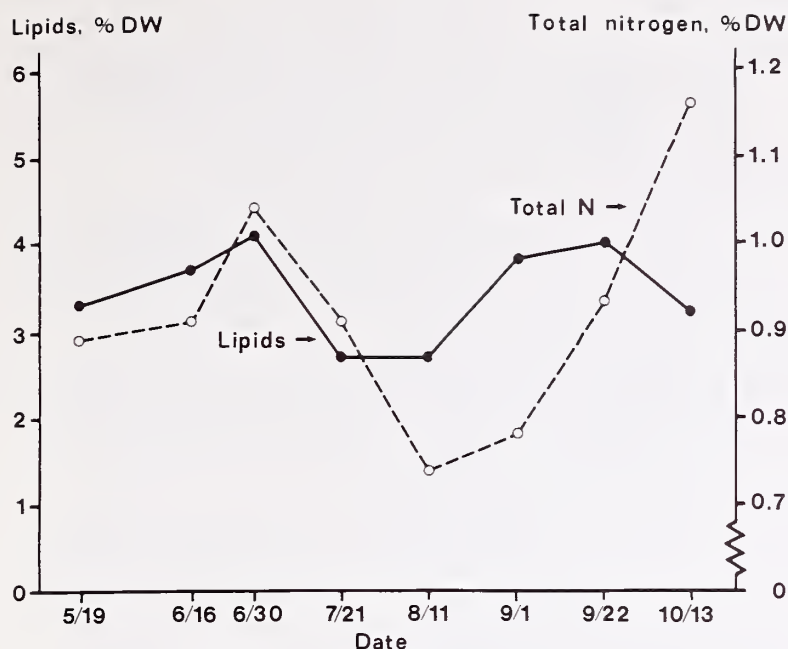


Figure 15.—Seasonal variations in concentrations of total nitrogen and saponifiable lipids in aspen roots.

Levels of lipids in aspen roots were much higher than those previously reported for tree roots (Kramer and Kozlowski 1960; Ziegler 1964). Generally, only small amounts of lipids are believed to be synthesized in roots of trees under normal conditions, with starch as the primary reserve material.

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This report reviews results of the author's research on the physiological mechanisms involved in the formation of adventitious shoots (suckers) in aspen roots. It describes origin and development of root suckers and presents the results of studies on the role of growth regulators and food reserves.

KEYWORDS: *Populus tremuloides*, trembling aspen, adventitious shoots, root suckers, clonal variation, auxins, cytokinins, gibberellins, abscisic acid, ethylene, carbohydrate reserves, lipids, nitrogenous compounds

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